Sugar Composition of Cucumber Cell Walls During Fruit Development

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ABSTRACT

The sugar composition of pickling cucumber cell walls was analyzed in seven cucumber sizes. Relative to total cell wall sugars, glucose did not change, while the other major neutral sugars, galactose, xylose and mannose, underwent relatively small declines during fruit development. The ratios of galacturonic acid/rhamnose and galacturonic acid/arabinose showed large increases during fruit growth, which indicated that pectin structure may change. Pectin methylation in the mesocarp tissue increased from 35 to 64% as cucumber fruit matured. A positive correlation was observed between the firmness of cucumber mesocarp tissue and the total amount of cell wall sugars in the fresh tissue. Cucumber peel tissue contained 4 to 5-fold higher sugar concentrations than either the mesocarp or endocarp tissues.

INTRODUCTION

A FIRM, CRISP TEXTURE is a primary quality attribute for all commercial cucumber pickle products. For processed fruit and vegetables, the physical and chemical structures of the cell walls are the main determinants of textural properties. In contrast to most fruits and vegetables which are harvested within a narrow range of maturity, cucumbers are harvested for processing from the time of pollination up through the period when the fruit weigh about 250g. Occasionally, specialty products are prepared from even larger fruit. For this reason it was of interest to determine the sugar composition of cucumber cell walls during the development of the fresh fruit and to determine the relationship between the sugars and the tissue firmness.

Studies of changes in cell wall polysaccharides during fruit ripening have been done for pears (Jermyn and Isherwood, 1956; Ahmed and Labavitch, 1980), Japanese pears (Yamaki et al., 1979), tomatoes (Gross and Wallner, 1979; Huber, 1983), and apples (Knee, 1973). Gross and Sams (1984) measured the noncellulosic neutral sugars at three developmental stages for 15 fruits, including cucumbers. Investigations of cell wall changes throughout fruit development have been done for Valencia oranges (Sinclair and Jolliffe, 1961) and strawberries (Knee et al., 1977).

Analysis of cell wall polysaccharides of fresh cucumbers has been done on fruit of a single size or maturity as a part of a larger study in a few cases. Fukushima and Yamazaki (1978) measured the certain pectin fractions and the degree of pectin methylation in fruits of a Japanese cultivar, Natsusairaku No. 3. Tang and McFeeters (1983) determined the pectin fractions and noncellulosic neutral sugars in 150g fruit of 'Chipper' cultivar. Voragen et al. (1983) determined pectin and the cellulosic and noncellulosic neutral sugars in European fresh-market cucumbers. Gross and Wang (1984) analyzed the noncellulosic sugars in 'Meadowist' cultivar. In their analysis of cucumber cell wall at three maturity stages, Gross and Sams (1984) did not find major changes in the neutral sugar composition. In contrast, galactose and arabinose were observed to decrease in other cucurbits such as melon, squash and muskmelon (Gross and Sams, 1984).

The objectives of this study were to analyze the sugar composition of the polysaccharides in the cell wall of cucumber mesocarp tissue during fruit development, to determine the relationship between the cell wall carbohydrate composition and mesocarp tissue firmness and to compare the mesocarp cell wall sugar composition with the composition of the peel and endocarp tissue cell walls of the cucumber fruit.

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MATERIALS & METHODS

Chemicals

Alditol acetate sugar derivative standards were purchased from Supelco, Inc. (Bellefonte, PA). Inositol, sodium borohydride, 1-methylimidazole, and dimethylsulfoxide were obtained from Sigma Chemical Company (St. Louis, MO). Acetic anhydride was from Baker Chemicals (Phillipsburg, NJ), and 3,5-dimethylphenol was from Aldrich Chemical Company (Milwaukee, WI).

Sampling

Cucumbers ('Calypso' cultivar) were obtained from the third picking of an irrigated experimental plot when the average fruit diameter was about 45 mm. All fruit were harvested on the same day except for the ripe fruit which were harvested from the same plot 25 days later. Fruit diameter was measured with a hand caliper to the nearest 0.1 mm. The fruit were classified by diameter to the following size ranges: #1B, 19.1 to 25 mm; #2A, 25.1 to 32 mm; #2B, 32.1 to 38 mm; #3A, 38.1 to 44 mm; #4, 51 to 55 mm; #5, 64 to 71 mm. Ripe fruit were designated #6 in this study. They were selected on the basis of a weight of >775 g and an endocarp pH of <4.4 (McFeeters et al., 1982). Sizes 1B through 4 are commercially defined sizes. Fifteen fruit of sizes 1 to 4 and 10 fruit of sizes 5 and 6 were randomly assigned to each of two lots. The weight and length of each fruit were determined. Peel, mesocarp and endocarp tissues from the cucumbers were separated, weighed and frozen until analysis.

In the previous year, similar sampling of the same cultivar was done except that only mesocarp tissue from sizes 2A, 3A, 4, 5, and 6 were analyzed.

Firmness measurements

Firmness of the mesocarp tissue of each fruit was determined by measuring the maximum penetration force required to puncture the tissue on an Instron UTM (Thompson et al., 1982). A single punch with a 0.315 cm diameter, flat-tipped plunger was made in the center of one mesocarp carpel section of a 7 mm thick slice taken from the middle of the fruit. A 2-kg compression force transducer was used. The crosshead speed and the chart drive speed were 200 and 500 mm/min, respectively. The maximum penetration force was recorded (Thompson et al., 1982).

Cell wall isolation

Cell walls were prepared from the mesocarp of each of the duplicate lots for each cucumber size. Walls were also isolated from the duplicate lots of peel and endocarp tissue (including seeds) from sizes 3A and 6. Mesocarp and endocarp tissue samples were blended in a Waring blender, then 120 g of the blended slurry was taken and immediately blended with 5 volumes of 95% ethanol. Peel tissue had to be mixed with an equal weight of water before blending. Five volumes of ethanol were added to 240g of the peel slurries. After filtration on

Whatman #4 filter paper, the alcohol-insoluble solids were resuspended once in 2.5 volumes of 70% ethanol and filtered, and then in 2.5 volumes of acetone and filtered again. The insoluble cell walls were dried at < 40°C in a vacuum oven and then placed in a desiccator over phosphorus pentoxide until analysis. The cell walls were collected quantitatively from the cucumber tissue (McFeeters and Armstrong, 1984) and weighed after drying so that concentrations of cell wall components could be expressed on a fresh weight basis as well as on the basis of the weight of isolated cell walls.

Sugar analysis

Triplicate samples were hydrolyzed and analyzed from each cell wall preparation. Neutral sugars in the samples were analyzed according to the procedure of Blakeney et al. (1983). This involved Saeman hydrolysis of the cell walls to monosaccharides, Na borohydride reduction of the monosaccharides to alditols, and acetylation of the alditols to give alditol acetate derivatives of the sugars for GLC. Dried cell wall (10 mg) was weighed into a 1.5 mL screw cap septum vial (Pierce Chemical Company, Rockford, IL). A small (4 mm) stirring bar was placed in the vial, then 125 μL of 72%, v/v, sulfuric acid was added. It was critical to mix the sulfuric acid with the cell walls immediately to obtain complete and uniform hydrolyses. This was accomplished by shaking the vial for 1 min on a dental amalgam mixer (Toothmaster Company, Racine, WI). After mixing, the vial was placed in a 25°C water bath for 45 min. Water (1.35 mL) was added to the vial and mixed with the sulfuric acid on a Vortex mixer. The vial was then placed in a boiling water bath for exactly 2 hr. After cooling, 320 μL concentrated ammonium hydroxide (15.9 M) was added to make the solution basic. Aliquots (200 μL) of the basic hydrolysates were put into 10 mL conical, Pierce Reacti-Vials, 20 μL of a 500 mM myo-inositol solution was added as an internal standard, then 1.0 mL of 2% w/v sodium borohydride in DMSO was added to the vial and incubated at 40°C for 90 min in a water bath. Excess borohydride was destroyed by adding 200 μL glacial acetic acid to the vial. After bubbling had ceased and the solution had cooled, 200 μL l-methylimidazole and 2.0 μL acetic anhydride were added to the vial. Acetylation was conducted for 20 min, then 5.0 mL water were added to decompose the remaining acetic anhydride. The vial was cooled and the alditol acetates were extracted three times with 1.0 mL aliquots dichloromethane. After each addition of dichloromethane, the vial was mixed on a Vortex mixer and the lower dichloromethane layer was removed with a Pasteur pipette. The dichloromethane from the three extractions was combined and evaporated to ~400 μL by standing in a hood. These solutions were either used for GLC analysis on the same day or kept in the freezer overnight and analyzed the next day.

Gas chromatographic analysis was done on a Hewlett-Packard 5890 GLC (Hewlett-Packard Company, Palo Alto, CA) equipped with a 30 meter × 0.25 mm DB-225 capillary column (J & W Scientific, Inc., Rancho Cordova, CA) and an FID detector. Injector and detector temperatures were 250°C. The oven temperature was held constant at 220°C. The flow rate of helium through the column was 1.1 mL/min. Nitrogen was used as the makeup gas. A 0.5 μL sample was injected into a split injector with the split ratio set at 13:1. Just prior to chromatography, 25 μL of 10 mM p-terphenyl solution was added to each sample. The p-terphenyl was used to check the recovery of myo-inositol after reduction and acetylation. Data were recorded and integrated with a Shimadzu CR3A integrator (Shimadzu Scientific Instruments, Inc., Columbia, MD). Sugar concentrations were calculated as the anhydro sugars relative to myo-inositol.

Pectin methylation analysis

Uronic acid and pectin methylation in the cell wall samples were analyzed as described previously (McFeeters and Armstrong, 1984) except that the Saeman hydrolysates (Blakeney et al., 1983) after ammonium hydroxide addition were used for the colorimetric analysis of the uronic acids. Use of these hydrolysates reduced the variability in the analysis compared to that obtained using the hydrolysis procedure of Scott (1979).

Calcium analysis

Calcium was analyzed by the colorimetric procedure of Gindler and King (1972).

Statistical analysis

Coefficients of variation for the analytical error of the individual sugar analyses were calculated by analysis of variance over all cucumber sizes. Linear regression analysis was used to determine if significant changes ($P \leq 0.05$) occurred in sugar ratios and firmness as fruit developed. Significant differences ($P \leq 0.05$) among peel, mesocarp and endocarp tissues for the galacturonic acid/rhamnose ratio and degree of pectin methylation in sizes 3A and 6 were determined by calculation of the LSD.

RESULTS

TABLE 1 shows the physical characteristics of the cucumbers used in this study. The proportion of peel tissue declined during fruit enlargement ($r^2 = 0.94$), as would be expected since the surface to volume ratio of the fruit declines. The proportion of mesocarp increased ($r^2 = 0.61$), while the endocarp tissue did not change ($r^2 = 0.13$). Though a number of possible variables could be utilized as an index of fruit development, fruit diameter was chosen because it is the commercial basis for size grading of cucumbers, and it varied over a convenient range.

Chromatography of neutral sugar derivatives gave baseline resolution of all peaks except rhamnose and fucose, which overlapped at about 5% of the peak heights for samples with equal sugar concentrations. After sugar derivatization, extraction and chromatography, the recovery of inositol was generally greater than 85%. This indicated that reasonably complete and uniform sugar recovery was achieved. Table 2 shows the retention time for sugars and p-terphenyl relative to the inositol hexaacetate retention time, the response factors relative to inositol hexaacetate and the coefficient of variation (CV) for each sugar analyzed. The CV for galacturonic acid analysis of the Saeman hydrolysates was 4.4%.

A primary reason for studying the composition of plant cell walls and the detailed structures of cell wall polysaccharides is to explain in chemical terms the physiological functions of the cell wall and the physical properties of plant tissues. Most efforts have been directed toward various aspects of polysaccharide structural analysis. However, it is possible that a physical characteristic such as tissue firmness may be as much influenced by the concentration of cell wall polysaccharides in a tissue as the detailed molecular structures of those polysaccharides. By combining the Blakeney et al. (1983) procedure for cell wall sugar analysis with quantitative recovery of cell wall material from cucumber tissues (McFeeters and Armstrong, 1984), it was possible to obtain reliable estimates of the concentrations of cell wall sugars on a fresh weight basis (Table 3). It can be seen that the total cell wall sugar concentration varied from 6.6 to 11.1 mg/g, while the concentrations of individual sugars varied from only 0.02 to 4.4 mg/g. Though the mesocarp firmness and total sugar concentration varied over a relatively small range, the change in fresh weight cell wall sugar concentration accounted for 73% of the firmness variation (Fig. 1). None of the individual sugars gave a better correlation than total sugar concentration. Neither the degree of pectin methylation nor the calcium ion concentration in the mesocarp tissue showed any significant correlation with tissue firmness.

Changes in the proportions of the eight sugars as a function of fruit diameter were determined by linear regression analysis (Fig. 2 and 3). The slope of the regression line was not significantly different from zero for glucose. Based upon the regression analysis, galacturonic acid increased 41% from size 1B to 6. Most of the increase occurred during the ripening period from size 5 to 6. It was the only sugar to increase relative to the total sugars during cucumber development. The other six sugars analyzed declined, ranging from 9% for mannose to 50% for rhamnose and arabinose.

Changes in the sugars associated with pectic substances were

Table 1—Physical characteristics of seven sizes of 'Calypso' cucumbers

Fruit size	1B	2A	2B	3A	4	5	6
Diameter, mm	22.7 ± 1.2	29.8 ± 1.7	35.4 ± 1.5	42.1 ± 1.7	53.8 ± 1.5	66.4 ± 3.2	81.2 ± 2.6
Weight, g	27 ± 5	55 ± 9	86 ± 10	136 ± 16	254 ± 29	419 ± 58	857 ± 66
Length, mm	80 ± 6	100 ± 8	114 ± 7	128 ± 9	152 ± 11	167 ± 13	218 ± 10
Mesocarp firmness, N	10.0 ± 0.8	9.3 ± 0.7	9.0 ± 0.9	8.7 ± 0.7	8.5 ± 0.6	7.8 ± 0.5	11.3 ± 1.4
Peel, % fruit wt	19.3	16.6	15.0	14.4	12.9	9.7	6.8
Mesocarp, % fruit wt	54.5	56.9	57.9	55.9	57.6	59.4	66.2
Endocarp, % fruit wt	26.2	26.5	27.1	29.8	29.5	31.0	27.0
Cell wall, mg/g fruit wt	12.4	13.5	11.7	10.2	9.2	9.7	14.3

Table 2—Retention times, response factors and coefficients of variation for GLC analysis of cell wall neutral sugars

	Relative retention time[a]	Relative response factor[b]	Coefficient of sugar variation, %[c]
Rhamnose	0.315	1.364	5.1
Fucose	0.329	1.151	7.5
Arabinose	0.389	1.500	5.0
Xylose	0.464	1.515	4.9
Mannose	0.815	1.075	4.9
Galactose	0.887	1.064	4.8
Glucose	0.954	1.145	3.6
p-Terphenyl	0.678	0.588	–

[a] Relative to inositol hexaacetate with an 18.5 min retention time.
[b] Relative to inositol hexaacetate.
[c] Analytical variation over all cucumber sizes.

of particular interest since the only sugar to increase was galacturonic acid, which is the major sugar in the main chain of pectic polymers. While galacturonic acid increased, substantial decreases occurred in rhamnose, a minor sugar in the main chain of pectin molecules, and arabinose, which is present in side chains of pectin in addition to other cell wall polymers. Figure 4 shows that significant increases in galacturonic acid/sugar ratios occurred during fruit development. Though not shown in Fig. 4, there was also a significant increase in the galacturonic acid/galactose ratio from 0.8 to 1.6. Since galactose was the second most abundant neutral sugar in the cell wall, most of the galactose is probably associated with polysaccharides other than the pectic polysaccharides. These changes may be indicative of changes in the structure of pectic polysaccharides during cucumber growth. In addition to changes in the ratios of sugars associated with pectic substances, the degree of pectin methylation increased from 35 to 64% as the fruit enlarged (Fig. 5).

In a more limited experiment in the previous year, analysis of the sugar composition of five sizes of cucumbers ('Calypso' cultivar) showed the same general pattern of changes. Sugars were present in the same relative proportions. Galacturonic acid increased, while rhamnose and arabinose declined such that there were consistent increases in the galacturonic acid/rhamnose and galacturonic acid/arabinose ratios. In addition, the degree of pectin methylation increased as the fruit enlarged (data not shown).

The peel and endocarp tissues were also analyzed for sizes 3A and 6. The most striking difference among the peel, mesocarp and endocarp tissues was that peel tissue contained much higher concentrations of cell wall sugars on a fresh weight basis than the other tissues (Table 4). The peel tissue had a 3.8-fold higher sugar concentration than the mesocarp in size 3A fruit and a 5.8-fold concentration in size 6. The size 6 fruit had higher cell wall sugar levels than the size 3A. Table 4 also shows the concentrations of individual sugars, the calcium concentrations and the weight of dry cell wall material isolated from the three tissues. Interesting points in these data were the extremely high concentration of xylose in the endocarp of the ripe (size 6) cucumbers, the low concentration of mannose in the endocarp and the fact that, in the size 6 endocarp tissue, the galacturonic acid/rhamnose ratio was only 15 compared to 49 for the mesocarp tissue and 40 for the peel (Fig. 6). The peel, mesocarp and endocarp galacturonic acid/rhamnose ratio was significantly different in each tissue in both cucumber sizes. Comparison of the degree of pectin methylation in the three tissues is shown in Fig. 7. The data showed very similar pectin methylation in the three tissues from the ripening fruit. However, in the size 3A fruit, pectin methylation was higher in the mesocarp than in the peel. The endocarp did not show a significant difference between either of the other two tissues. Calcium concentrations were highest in the peel tissues, with the greatest differences between the peel and the mesocarp tissue in the ripening fruit.

DISCUSSION

THE PROCEDURE of Blakeney et al. (1983) was a significant improvement over the earlier technique of Albersheim et al. (1967) for preparing alditol acetate derivatives of sugars since using 1-methylimidazole as an acetylation catalyst did not require evaporation of water and borate from the samples prior to acetylation. However, it was necessary to make a few modifications to the procedure to obtain consistent, reproducible results. Mixing the cell wall and 72% sulfuric acid with an amalgam mixer was essential for reproducible hydrolysis of the cell wall samples. The addition of 200 μL glacial acetic acid instead of 100 μl to decompose excess borohydride resulted in fewer and smaller reagent peaks in the final chromatograms. Three extractions with dichloromethane and then concentration of the samples to about 400 μL gave a higher final concentration of derivatized sugars and larger GLC peaks. Finally, we were able to provide a measure of quality control for the derivatization and concentration procedures by the addition of p-terphenyl to samples just prior to injection into the

Table 3—Concentration of cell wall sugars and calcium in fresh cucumber mesocarp tissue

Fruit size	1B	2A	2B	3A	4	5	6
				(mg/g Fresh mesocarp tissue)			
Galacturonic acid	1.67	2.00	1.78	1.65	1.60	1.67	3.52
Rhamnose	0.088	0.084	0.071	0.055	0.048	0.045	0.060
Fucose	0.035	0.041	0.036	0.030	0.029	0.023	0.035
Arabinose	0.37	0.37	0.32	0.24	0.20	0.19	0.27
Xylose	0.49	0.58	0.51	0.45	0.42	0.41	0.62
Mannose	0.33	0.41	0.36	0.32	0.29	0.29	0.45
Galactose	1.67	1.91	1.72	1.54	1.33	1.38	1.78
Glucose	3.10	3.65	3.22	2.96	2.70	2.93	4.39
Total sugars	7.75	9.04	8.02	7.26	6.61	6.93	11.12
Calcium	0.17	0.12	0.099	0.092	0.096	0.11	0.068

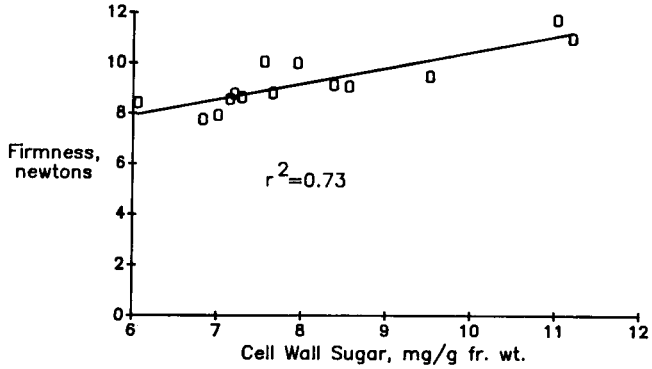

Fig. 1—Relationship between the sugar content of the cucumber mesocarp cell walls and the firmness of the tissue.

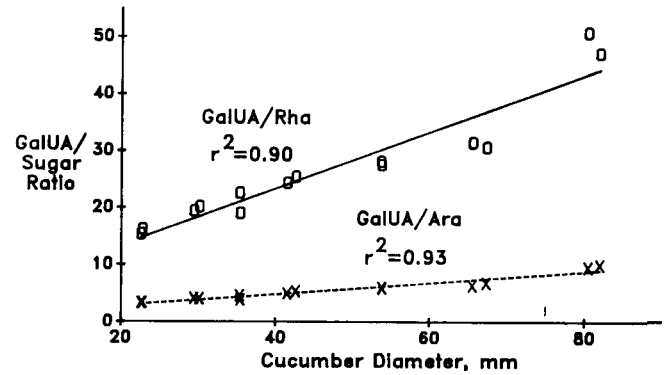

Fig. 4—Changes in the galacturonic acid/rhamnose and galacturonic acid/arabinose molar ratios in mesocarp cell walls during development of the cucumber fruit.

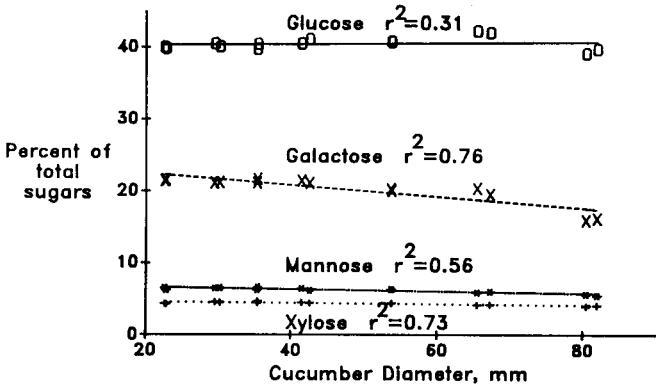

Fig. 2—Changes in glucose, galactose, xylose, and mannose compared to the total sugars isolated from cucumber mesocarp tissue cell walls during fruit development.

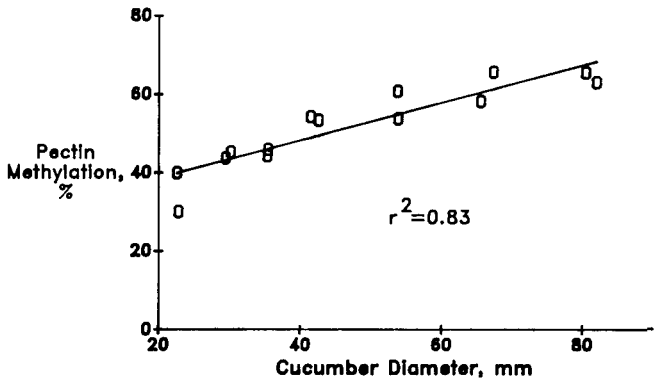

Fig. 5—Changes in the degree of pectin methylation in the mesocarp tissue during development of the cucumber fruit.

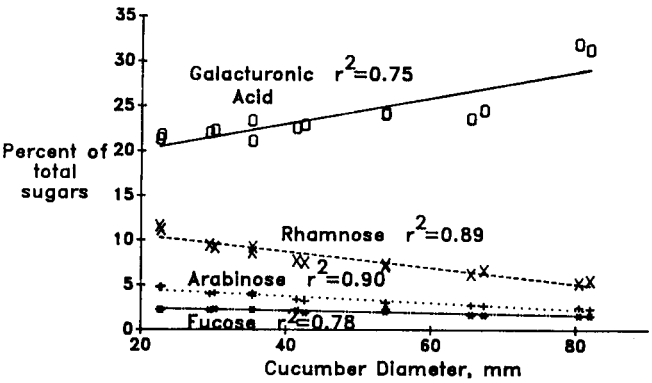

Fig. 3—Changes in galacturonic acid, arabinose, rhamnose, and fucose compared to the total sugars isolated from cucumber mesocarp tissue cell walls during fruit development. The rhamnose concentrations were multiplied by 10, and the fucose concentrations were multiplied by 5.

gas chromatograph. This compound did not require derivatization for chromatography and gave a peak well separated from sugar peaks. The response factor of p-terphenyl relative to an inositol hexaacetate standard was determined so that the recovery of added inositol from each derivatized sample could be calculated. As a result, any problems with the derivatization or extraction steps in the analytical procedure were quickly apparent.

The relative abundance of sugars in cucumber cell walls in this study was consistent with previous reports (Voragen et al., 1983; Tang and McFeeters, 1983; Gross and Sams, 1984). Though all of the neutral sugars except glucose declined during fruit growth, the major sugars, glucose, galactose, xylose and mannose, remained nearly constant relative to each other during the period that the weight of the fruit increased over 30-fold. The ratios of these sugars were 9.4:4.7:1.8:1.0 glucose:galactose:xylose:mannose. This could be an indication that the polysaccharides containing these sugars are synthesized in a coordinated manner by the cells. Fucose declined by 31% during cucumber development. It is a minor cell wall sugar that has been found in the side chains of xyloglucans and in rhamnogalacturonan II in dicot cell walls (McNeil et al., 1984).

The changes in galacturonic acid/sugar ratios have significant implications for possible changes in the structure of pectic polysaccharides during fruit development. Rees and Wight (1971) showed that the stereochemistry of a rhamnose residue in a galacturonan chain forced a kink in the polymer whether the rhamnose was in the alpha or beta configuration. If the current concept that rhamnose is located primarily in the main chain of polygalacturonans (Darvill et al., 1980) is correct, these data suggest that the shape of the pectin molecules must change substantially during cucumber development. In immature fruit, the molecules would have frequent bends or kinks, while the pectin chains would have fewer kinks and become more rod-shaped as the fruit matured. This possibility needs to be investigated by determining the structural characteristics of the pectin from mature and immature fruit. Gross and Sams (1984) observed a decline in rhamnose during the maturation of several fruit, including cucumbers. However, they did not measure the uronic acid content of the cell walls.

Galactose and arabinose are generally the most abundant neutral sugars in isolated pectins (Voragen et al., 1983), but they also occur in other cell wall polysaccharides (Darvill et al., 1980). Since the galacturonic acid/sugar ratios increased during cucumber growth, this could indicate that the pectic

Table 4—Sugar and calcium distribution in the peel, mesocarp and endocarp tissues of size 3A and size 6 cucumbers

Fruit size	3A			6		
tissue	Peel	Mesocarp	Endocarp	Peel	Mesocarp	Endocarp
		(Mole %)			(Mole %)	
Galacturonic acid	24.6	20.8	18.1	35.8	29.3	14.2
Rhamnose	1.11	0.84	1.04	0.90	0.60	1.15
Fucose	0.71	0.45	0.45	0.49	0.36	0.29
Arabinose	6.5	4.0	8.8	5.8	3.0	4.6
Xylose	8.7	7.7	7.0	6.9	6.8	26.7
Mannose	3.9	4.4	1.9	3.1	4.0	1.2
Galactose	14.4	21.2	21.9	11.2	16.1	10.2
Glucose	40.1	40.6	40.8	35.9	39.7	41.7
Total sugars, μmoles/g fruit wt	172.9	45.0	38.6	394.9	68.2	75.6
Calcium, μmoles/g fruit wt	6.8	2.3	1.4	18.0	1.7	1.4
Cell wall, mg/g fruit wt	554.0	10.2	10.8	102.7	14.3	32.7

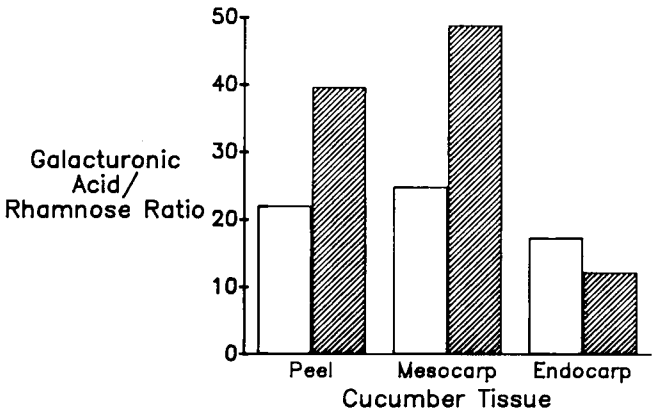

Fig. 6—Comparison of galacturonic acid/rhamnose molar ratios in cucumber cell walls isolated from peel, mesocarp and endocarp tissues of size 3A □ and size 6 ■ cucumbers. LSDs among tissues were 1.0 for size 3A and 4.0 for size 6 fruit.

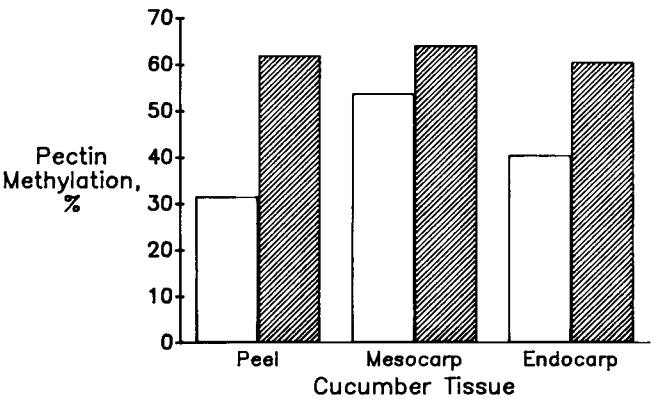

Fig. 7—Comparison of the degree of pectin methylation in cucumber cell walls isolated from peel, mesocarp and endocarp tissues of size 3A □ and size 6 ■ cucumbers. LSDs were 17.2% for size 3A and 5.7% for size 6 fruit.

substances in larger fruit have fewer or shorter neutral sugar side chains, but it could also mean that the amounts of these sugars in the hemicellulose fraction of the wall declined. Galacturonic acid, rhamnose and arabinose all declined during ripening of pears (Ahmed and Labavitch, 1980; Jermyn and Isherwood, 1956). DeVries et al. (1981) did not find substantial differences in the neutral sugar composition of pectin isolated from ripe and unripe apples.

Cucumbers appear to be unusual in the fact tnat the degree of pectin methylation in the mesocarp increased during fruit development from 35 to 64%. With the exception of orange peel, which was found to increase from 62 to 80% methylation during fruit development (Sinclair and Jolliffe, 1961), pectin esterification generally remains constant as in apples (Woodmansee et al., 1959; DeVries et al., 1981) or decreases, as occurs in avocado and pear (McCready and McComb, 1954), tomato (Woodmansee et al., 1959), peaches (Sterling and Kalb, 1959), and cherries (Davignon, 1961).

The positive correlation between the fresh weight concentration of cell wall sugars and cucumber firmness suggested that, at least as a first approximation, firmness could be predicted by the total cell wall sugar content. It would be most interesting if such a simple relationship were generally true, considering the complexity of cell wall polysaccharides (McNeil et al., 1984) and the variability in the sugar compositions among different fruit (Voragen et al., 1983). However, there is only one study on ripening of pears (Ahmed and Labavitch, 1980) in which cell wall sugar concentrations were determined on a fresh weight basis and tissue firmness was measured. We have calculated a significant positive relationship between firmness and sugar concentration using those data. Further studies of this kind, perhaps comparing tissues among several species, need to be done to determine if this is a general relationship. Goffinet (1977) attempted to relate anatomical characteristics of the cucumber mesocarp tissue to the texture of different cucumber cultivars. The average cell number was the only characteristic he found that showed a significant relationship to texture.

Little attention has been paid to the differences in cell wall composition in different parts of a plant. Nevins et al. (1967) measured the neutral noncellulosic sugars in the leaves, roots, first internodes, and hypocotyls of 18-day old kidney beans, mung beans and soybeans. They found differences among the tissues, but not among the same tissues in different species. The high concentrations of cell wall in the peel tissue, in addition to the cutin layer on the outside of the fruit, may be important in determining the barrier properties of the cucumber skin. This may be significant to the protection of whole cucumbers against softening by fungal enzymes during fermentation (Buescher et al., 1979, 1981). The differences in the galacturonic acid/rhamnose ratio among the peel, mesocarp and endocarp tissues and the differences in the degree of methylation in the size 3A tissues indicated that the structure of the pectic substances could vary among the different fruit tissues. The significance of these modifications to the structural and textural characteristics of the fruit remains to be determined. The most obvious difference in the proportions of individual sugars in the different tissues was the large accumulation of xylose in the size 6 endocarp.

SUMMARY & CONCLUSIONS

ANALYSIS OF CELL WALLS from seven sizes of a pickling cucumber cultivar has resulted in several new findings concerning the structure of the cell wall and its relationship to tissue firmness. The major neutral sugars in the cell wall poly-

saccharides, glucose, galactose, xylose, and mannose, showed relatively small changes during development. They maintained rather constant ratios relative to each other, which could be an indication of coordinated accumulation as fruit grow. The largest changes occurred in those sugars associated with the pectic substances. The increase in the galacturonic acid/rhamnose ratio suggested that pectin polymers in mature fruit have fewer main chain bends or kinks than those in immature fruit. The increases in the galacturonic acid/arabinose and galacturonic acid/galactose ratios could mean that pectin in large fruit had fewer or shorter side chains. However, since these sugars are known to be associated with polysaccharides other than the pectin substances, this is a less certain possibility than changes in the main chain structure. Data have not been previously reported which suggest such developmental changes in pectin structure in fruit. In addition to changes related to sugars in pectic polymers, the degree of methylation of pectin also increased from 35 to 64% during fruit growth.

It has not been common in studies of the composition of plant cell walls to report data on the basis of fresh tissue weight. However, in this study a positive correlation was observed between the concentration of total cell wall sugars and mesocarp tissue firmness, which accounted for 73% of the firmness variation. This relationship indicated that attempts to relate the physical characteristics of plant tissues to chemical structure should include an evaluation of the contribution of cell wall sugar concentration to physical properties.

The most striking finding regarding the sugar composition of different tissues in the cucumber also related to the fresh weight concentration of sugars. The concentration of cell wall sugars was much higher in peel tissue than in mesocarp or endocarp tissue. This high cell wall polysaccharide concentration could be an important factor in the barrier properties of the cucumber peel. There were also differences in the relative amounts of certain sugars in the peel, mesocarp and endocarp tissues and in patterns of pectin methylation. These results were in line with earlier observations in other plants that plant tissues can differ in the relative amounts of their cell wall sugars.

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